

US-PAT-NO: 5766894

DOCUMENT-IDENTIFIER: US 5766894 A

TITLE: Production of vitamin B_{sub}.6 by
fermentation

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Brief Summary Text - BSTX (7):

For carrying out the present invention, microorganisms belonging to the genus *Rhizobium* are incubated in aqueous culture medium containing assimilable carbon sources, digestible nitrogen sources, inorganic salts and other nutrients necessary for the growth of the microorganism. As the carbon source, for example, glucose, fructose, lactose, galactose, sucrose, maltose, starch, dextrin or glycerol may be employed. As the nitrogen source, for example, peptone, soybean powder, corn steep liquor, meat extract, ammonium sulfate, ammonium nitrate, urea or mixtures thereof may be employed.

Further, as the inorganic salts, sulfates, hydrochlorides or phosphates of calcium, magnesium, zinc, manganese, cobalt and iron may be employed. And, if necessary, conventional nutrient factors or an antifoaming agent such as animal oil, vegetable oil or mineral oil can also be added. The pH of the culture medium is suitably from about 5.0 to about 9.0, preferably from about 6.5 to about 7.5. The cultivation temperature is suitably from about 10.degree. to about 40.degree. C., preferably from about 26.degree. to about 30.degree. C. The cultivation time is suitably from about 1 to about 14 days, preferably from about 2 to about 7 days. In the cultivation, aeration and agitation usually

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Microbiology

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Research interest and current activities

My research interest centres around the physiology of rhizobia. Rhizobia are soil bacteria and are ubiquitous in nature. These enter into symbiotic interaction with cognate legume host and incite production of nodules in the roots/stems of the host plant. Within the nodules the bacteria become transformed into bacteroids and fix atmospheric N₂ at the expense of plant photosynthate.



Our research works have revealed the following:

Although the enzymes of the Embden-Meyerhoff-Parnas (EMP and the Entner-Duodoroff (ED) pathways for glucose metabolism are present in the cultured cells of rhizobia Phytochemistry, 1987, 26, 85; Indian J. Biochem. Biophys., 1989, 26, 120), these are inducible in nature and subject to catabolite repression (Current Microbiol., 1993, 26, 247). Cultured cells of rhizobia contain cytochromes c, b, aa₃, o and a soluble CO-reactive haemoprotein, P-428, in them.

During acetate metabolism by strains of rhizobia glyoxylate pathway is induced and the induction of isocitrate lyase, the key enzyme of the pathway, is under the control of cAMP (J.Gen.Appl. Microbiol., 1992, 38, 417).

Several strains of rhizobia have evolved a mechanism of acquisition of iron through the production of siderophores. Siderophore production and its interaction with other metal ions revealed the role of siderophore in transport of metal ions through cell membrane (Microbiology, 1994, 140, 2811; Current Microbiol., 2000, 41, 5).

Some strains of rhizobia are able to solubilize insoluble phosphates including rock phosphates. 2-ketogluconate was found to be a major factor in the solubilization process (J.Gen.Appl.Microbiol., 1990, 36, 81).

Our current activities also include studies on biocontrol of wilt disease of tomato by introduction of plant growth promoting rhizobacteria (PGPR) in the rhizosphere.

Name of group members

- Dr. Anup K. Haldar
- Mithu De
- Anasuya Ray

Important publications (ten)

1. Roy N and Chakrabarty P K. 2000. Effect of aluminum on the production of

siderophore by Rhizobium sp. (*Cicer arietinum*). *Current Microbiology*, 41, 5-10.

- 2. Datta J , Maiti A K, Modak D P, Chakrabarty P K, Bhattacharyya P. and Ray PK. 2000. Metabolism of g-hexachlorocyclohexane by *Arthrobacter citreus* strain BI-100: identification of metabolites. *J. Gen. Appl. Microbiol.* 46, 59-67.
- 3. Halder A K and Chakrabarty P K. 1995. Constitutive nitrate and nitrite reductase activities of Rhizobium in relation to denitrification. *J. Basic Microbiol.* 35, 233-239.
- 4. Roy N, Bhattacharyya P and Chakrabarty PK. 1994. Iron acquisition during growth in an iron-deficient medium by Rhizobium sp. isolated from *Cicer arietinum*. *Microbiology*. 140, 2811-2820.
- 5. Roy N, Chowdury A, Roy S and Chakrabarty PK. 1994. Effect of cyanate on nitrate reduction by Rhizobium meliloti SU47. *J. Basic. Microbiology*, 16, 259-264.
- 6. Mandal NC and Chakrabarty PK. 1993. Succinate mediated catabolite repression of enzymes of glucose metabolism in root-nodule bacteria. *Current Microbiol.* 26, 247-251.
- 7. Mandal NC and Chakrabarty, PK. 1992. Regulation of enzymes of glyoxylate pathway in root-nodule bacteria. *J. Gen. Appl. Microbiol.* 38, 417-427.
- 8. Halder AK, Mishra AK, Bhattacharyya P and Chakrabarty PK. 1990. Solubilization of rock phosphate by Rhizobium and Bradyrhizobium. *J. Gen. Appl. Microbiol.* 36, 81-92.
- 9. Chakrabarti SK, Halder AK and Chakrabarty PK. 1990. Relatedness among Rhizobium species. *J. Gen. Appl. Microbiol.* 36, 47-54.
- 10. Mandal NC and Chakrabarty PK. 1989. Enzymes of carbohydrate metabolism in fast-growing Rhizobium grown on hexoses or succinate. *Indian J. Biochem. Biophys.* 26, 120-122.

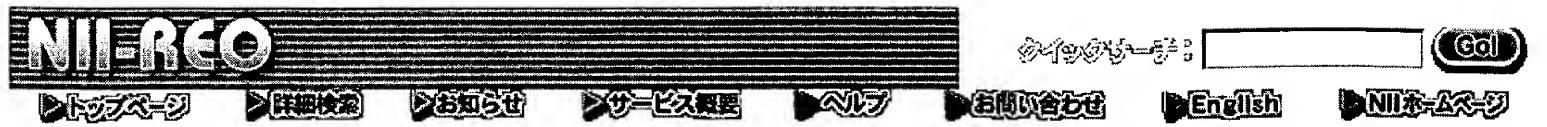
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Iron requirement and siderophore production in *Rhizobium ciceri* during growth on an iron-deficient medium

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抄録

Under conditions of iron limitation many rhizospheric bacteria produce siderophores, ferric iron-specific ligands, which may enhance plant growth by increasing the availability of iron near the roots. Thirty-five strains of *Rhizobium ciceri*, specific to chickpea (*Cicer arietinum* L.), were screened for their ability to grow on iron-deficient medium and to produce siderophores. Maximal growth of all strains previously depleted in iron was obtained in medium containing 5 to 10 μ m of ferric iron. When iron limitation was achieved by the addition of 2,2-bipyridyl or EDDHA [ethylene diamine di(o-hydroxyphenyl) acetic acid] to the medium, only two strains were able to scavenge iron and grow. Siderophore production by these two strains was detected by the Chrome Azurol S assay (CAS), a universal test for siderophores. No hydroxamate-type siderophores were detected in the supernatants of *Rhizobium ciceri* cultures. However, some strains secreted salicylic acid and 2,3-dihydroxybenzoic acid as phenolate-type siderophores. Addition of ferric iron to the culture medium increased growth yield significantly but depressed the production of siderophores. Although these compounds are produced in response to iron deficiency, nutritive components of the culture medium significantly affected their production. It seems that CuII, MoVI and MnII ions bound competitively with iron to siderophores, resulting in a 34 to 100% increase in production.

キーワード

2,3-Dihydroxybenzoic acid, Iron deficiency, *Rhizobium ciceri*, salicylic acid, siderophore production

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